Comparison of ARIES® Group A Streptococcus (IUO) Assay with Culture for Laboratory Detection of Acute Pharyngitis

ECCMID 2017

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RESULTS

Comparison of ARIES GAS IUO Assay with Culture

Table 1. Pre-Clinical Study Data

<table>
<thead>
<tr>
<th>Culture</th>
<th>GAS POS</th>
<th>GAS NEG</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARIES GAS POS</td>
<td>51</td>
<td>5</td>
<td>56</td>
</tr>
<tr>
<td>GAS NEG</td>
<td>0</td>
<td>128</td>
<td>128</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
<td>133</td>
<td>184</td>
</tr>
</tbody>
</table>

Table 2. Performance of ARIES GAS IUO Assay

<table>
<thead>
<tr>
<th>Value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPA</td>
<td>51/51 (100%)</td>
</tr>
<tr>
<td>NPA</td>
<td>128/133 (96.2%)</td>
</tr>
</tbody>
</table>

Table 3. ARIES GAS IUO Assay Performance -- Post Discrepant Analysis

<table>
<thead>
<tr>
<th>Value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPA</td>
<td>95/96 (100%)</td>
</tr>
<tr>
<td>NPA</td>
<td>128/128 (100%)</td>
</tr>
</tbody>
</table>

* 5 discrepant: ARIES POS (2) + 33.8, 38.5, 34.5, 33.9 & 33.8 vs Culture NEG All 5 were confirmed as POS by sequencing

CONCLUSIONS

• Data from the pre-clinical study demonstrates that ARIES GAS IUO Assay is a highly sensitive and specific assay for the detection of S. pyogenes from throat swab specimens.

• The ARIES assay is easy to set up with results for up to 12 specimens available within 2 hours of specimen receipt.

• The sample to result automation and random access of two modules (6 tests on each module) on the ARIES instrument provides flexibility to setup ARIES GAS assay in two batches.

• The universal amplification protocol for DNA and RNA targets on the ARIES platform makes it an ideal automated molecular instrument for laboratories due to the availability of many clinical assays (HSV1 & 2, Flu A/B, RSV, Group B streptococcus) and the ability to test lab developed assays.

ABSTRACT

Background: Group A streptococcus (GAS) is the leading bacterial cause of acute pharyngitis in children and adults. Rapid detection of GAS helps with timely initiation of antimicrobial therapy to avoid the severe, albeit less frequent post infectious sequelae. Rapid antigen tests for GAS have low sensitivity warranting culture confirmation, especially in children. ARIES® Group A Streptococcus Investigational use only assay (ARIES GAS IUO assay) is a rapid molecular test for detection of GAS from throat swab specimens. The assay is performed on the Luminex® ARIES® system, a fully integrated, sample-to-answer platform that performs real-time PCR assays.

Materials/methods: The performance of the ARIES® GAS IUO assay in development was compared with culture for GAS as reference method. Throat swab specimens collected by Copan ESwab™ and suspended in Liquid Amies were included in the study. One aliquot of the Liquid Amies suspension was cultured in GAS selective agar and Sheep blood agar, incubated aerobically up to 48 hours and beta-hemolytic colonies were identified by biochemical, latex antigen and MALDI-TOF analysis. A second aliquot was tested by the ARIES® GAS molecular assay and results were compared.

Results: Among the 184 throat swab specimens cultured, 51 were positive for GAS. ARIES® GAS IUO assay detected GAS in all of the 51 culture positive specimens and 5 additional specimens that were originally culture negative. The range of the cycle threshold values for the ARIES® GAS IUO assay for all positive specimens was 20.9 to 39.5 and 31.8 to 38.8 for the 5 specimens positive by ARIES® GAS IUO assay only. The positive and negative predictive agreement of ARIES® in comparison to GAS culture was 100% (95% CI: 93%-100%) and positive and negative predictive agreement of ARIES® improved to 100% (95% CI: 93.6%-100%) and 100% (95%CI:97.1%-100%) respectively. Bi-directional sequencing of a target other than the ARIES® GAS IUO assay confirmed GAS sequences in all of the 5 discrepant specimens. Following discrepant analysis the positive and negative predictive agreement of ARIES® improved to 100% (95% CI: 93.6%-100%) and 100% (95%CI:97.1%-100%) respectively.

Conclusion: ARIES® GAS IUO assay is a rapid, automated molecular assay that is highly sensitive and specific for GAS detection directly from throat swabs transported in Liquid Amies. The high sensitivity of the ARIES® GAS IUO assay eliminates the need for backup culture. Implementation of this test has the potential to improve appropriate use of antibiotics and reduce spread of disease.

INTRODUCTION

This was an R&D pre-clinical study to evaluate the performance of the ARIES GAS IUO Assay to reference culture method. Prospectively collected, fresh, left-over de-identified clinical specimens that meet the inclusion/exclusion criteria were blinded, and evaluated for the detection of S. pyogenes.

An exemption from the requirements for Informed Consent was granted by the site IRB to include the left-over clinical specimen in the study. A Nylon Flocked Swab with 1mL modified Liquid Amies (E Swab™ or Opti-Swab™) and left over specimen with a minimum of 375 ul was enrolled in the study. Two aliquots were made. Aliquot 1 was shipped to the reference laboratory within 24 hours for Group A streptococcus culture and setup within 48 hours and Aliquot 2 was compared with culture for GAS as reference method. Throat swab specimens (250ul) was stored at 2-8C and tested on ARIES instrument with in 72 hours of collection.

ARIES GAS IUO Assay Testing: The workflow of the Group A streptococcus realtime PCR assay on ARIES instrument is shown in figure 1. The ARIES GAS assay cassettes were loaded on the carrier (figure 2) and inserted in to one of the two modules in the ARIES instrument (fig 3).

ARIES GAS IUO Assay Testing: Culture: S. pyogenes media was inoculated in Group A streptococcus selective agar with 5% sheep blood (SSA) and Tryptic Soy agar with 5% sheep blood (TSA) and incubated for up to 48 hours in anaerobic conditions using BD Gas Pak EZ generation system. Beta hemolytic colonies were subcultured and identified by MALDI-TOF analysis.

ARIES GAS IUO Assay Testing: The primary objective of this pre-clinical study is to confirm that the specimen processing and reference culture comparison method are appropriate prior to execution of a clinical trial.

ARIES GAS IUO Assay Testing: The ARIES Group A Streptococcus Extractor Kit (Extractor) is an automated test for qualitative detection of S. pyogenes from patients suspected of having pharyngitis consistent with a group A Streptococcus infection. The assay is intended for use with throat swab specimens from symptomatic individuals. The ARIES Group A Streptococcus Extractor Kit is performed on the Luminex ARIES system, an automated platform that performs sample preparation and real-time polymerase chain reaction (PCR) for the detection of target-specific DNA. The assay reagents are supplied in disposable fluidic cartridges and include sample preparation reagents, assay controls, and PCR reagents. The ARIES Group A Streptococcus Extractor Kit detects S. pyogenes as well as a sample processing control.

ARIES GAS IUO Assay Testing: The ARIES GAS IUO Assay is a real-time PCR based qualitative in vitro diagnostic test for the direct detection of S. pyogenes (Group A β-hemolytic Streptococcus) in throat swab specimens from patients with signs and symptoms of pharyngitis.

ARIES GAS IUO Assay Testing: The primary objective of this pre-clinical study is to confirm that the specimen processing and reference culture comparison method are appropriate prior to execution of a clinical trial.

MATERIALS AND METHODS

Background: Comparison of ARIES GAS IUO Assay with Culture

Materials/methods: This was an R&D pre-clinical study. This was an R&D pre-clinical study. The primary objective of this pre-clinical study is to confirm that the specimen processing and reference culture comparison method are appropriate prior to execution of a clinical trial.

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